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SEPARATION AND IDENTIFICATION OF A NUMBER OF UNSATURATED FATTY ACIDS FROM DIFFERENT TYPES OF ALGAE

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Abstract

Recently, the interest in sustainable development is one of the most important fields of science and research because it aims to improve the life environment for all living organisms in general and for humans in particular. One of the areas of sustainable development is maintaining the integrity of the environment, reducing pollution, preserving energy sources and using clean energy from sustainable environmentally friendly sources. Algae represent the most important sustainable sources that succeed in achieving these goals, in addition to being a sustainable and renewable source for efficiently producing biofuels, they produce living mass that is used in the production of compounds that are used in various medical, industrial and agricultural industries. Besides, during their growth, they reduce pollution by producing oxygen and reducing carbon dioxide used in Photosynthesis to build different compounds and their high ability to absorb pollutants from various heavy metals, especially as well as nitrogen and phosphorous in the case of their increase in the developing environment in it, and the requirements for their growth are inexpensive and economical. The results showed, after separating of the algal oils by the Soxhlet extraction method with using hexane as a solvent for extraction, the superiority of the genus Westiellopsis in terms of the amount of lipids produced from biomass, amounting to 20%, followed by Gloeocapsa 18.5 %, while the unbranched filamentous alga Oscillatoria recorded 6.1% lipids. Therefore, the importance of composition of these oils from fatty acids represent important feature that is due to it in addition to the percentage of oil content in algae, the quality and quantity of these fatty acids differed between the species under study. The fatty acids that are preferred to be present in a higher percentage than the rest of the species are saturated and monounsaturated acids, as the first amounted to 2.14% and the second to 0.128% in Westiellopsis, so the percentage of desired fatty acids became 2.268% of the total diagnosed fatty acids 3.141%, , while Gloeocapsa oil showed the best percentage of preferred fatty acids for biodiesel (86.673%) which were composed from 0.969 % desired biodiesel fatty acids(saturate and monounsaturated fatty acids) , with a quantity of oils close to the. 0.515 % and best type in this study as indicated above. highest USFA in Westiellopsis that results in highest DU with corresponding to lowest % USFA/SAFA as well as lowest CN, while the equilibrium of DU for Gloeocapsa and Oscillatoria showed owning an equilibrium CN too.

Keywords: Cyanobacteria, Fatty Acids, Biodiesel DU, GC, Biodiesel CN.

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Introduction

Algae (macroalgae) are macroscopic photosynthetic organisms ,while (microalgae) are microscopic , both of them can be present in water environment (fresh or marine water) and also use CO_2 and nutrients for growth. The more advantages for algae are microalgae because they are the fastest growing organisms and can complete an entire growing cycle every few days [1]. Microalgae are undefiled organisms spot announcement as diatoms (Bacillariophyceae) , green algae (chlorophyceae) , golden cyanobacteria (cyanophyceae). They shot a not far from stretch of physiological and biochemical donation and their coalition unique judge (50-70%) protein , there than (30%) lipids , and the vitamins (B , E , D) etc. incomparison far adaptation plants or animals. [2] eventually , macroalgae (the vanquish multicellular algae , the seaweeds , trust a unheard-of bring about of eukaryotic , multicellular (in inches or more) algae as brown algae (phaeophyate) , red algae (rhodophyte), and green algae (chlorophyate). ----- evolutionary and biochemical similarities to higher plants [3]. Microalgae offer and attractive choies to generate renewable and sustainable biofuels. Biofuels produced from algae have lower environmental impact compared with biofuels produced from crops and do not compete with food supply [4] . Microalgae are regarded as valuable bioresources because, depending on their biological habitat, they have the capacity to accumulate protein, carbohydrates, and other high-value compounds inside the cell. The use of algae as a feedstock for biofuel and other industrial byproducts is possible. As a result, algae have potential uses in the biofuel, cosmetics, nutraceuticals, and pharmaceutical industries [5].

Cyanobacteria are autotrophic prokaryotes that can perform oxygenic photosynthesis and it considered as microalgae .It a diverse group and can grow rapidly due to their simple structure . they are unicellular or multicellular have ability to convert solar energy to chemical energy by photosynthesis . Significant progress was done The biosynthesis of various green fuels and chemicals directly from CO2 in the cyanobacterial chassis has shown the enormous potential of the renewable cyanobacterial systems in the production of green fuels and chemicals in the future [6].

The development of different kinds of biofuel extraordinarily relies upon feedstock accessibility and the carried out mechanical choices . microalgae biomass creation represents 65-85% of the general expense of biofuel fabricating among the bioenergy feed stocks , areal lipid (biomass efficiency green growth is quite possibly of the greatest. Biodiesel creation from green growth as a rule includes the transformation of lipids from green growth through roundabout transesterification in two stages . the initial step commonly is dewatering of green growth and drying of green growth biomass , followed by the extraction of lipids that are then transesterified for the blend of biodiesel , lipid extraction strategies are one of these : mechanical (press/expeller , globule processing , electroporation , salvation) , physical (ultrasonic , microwave , beat electric field , lyophilization warm) , synthetic (solvents , Soxhlet extraction , supercritical liquids) , and natural (chemicals) applications direct transesterification (D-TE) is a one stage process in view of the synergist change of lipids of algal biomass to Notorieties or biodiesel which is 15-20% a bigger number of productive than the backhanded cycle. [7,8].

The lipid content in algae and microalgae is between 8% and 40% of biomass depending on its strain as well as affected with culture conditions [9].

The cyanobacteria in particular offer an opportunity to generate valuable and useful products and in the same time decreasing the amount of co2 . [10].

Microalgae are capable of synthesizing a range of biofuels such as : lipids and carbohydrates that considered as substrates for biofuel production in additional to proteins that surve both as food and feed . the residual biomass of microalgae could be further fermented to ethanol or biogas by yeasts or anaerobic bacteria [11] .

Cyanobacteria and microalgae have several aspects for biofuel production such as : oxygenic photosynthesis, high per-acre productivity, non-food based feedstock, growth on unproductive, non-arable land, use of a wide range of water sources, and generation of useful byproducts in addition to biofuels are a few examples. Biofuels currently available include biogas, bioethanol, biodiesel, and biohydrogen [12,13].

Biodiesel has turned into a critical source as a replacement fuel and is making its place as a key future sustainable power source. As an elective fuel for diesel motors, it is turning out to be progressively significant because of reducing petrol holds and the ecological results of fumes gases from petrol fuelled engines.[14]

Petroleum products are non-sustainable power assets. Albeit, these powers are contributing to a great extent to the world energy supply, their creation and use have raised ecological worries and political discussions. It has been shown that 98% of fossil fuel byproducts are come about because of petroleum derivative ignition [15] . Biodiesel is characterized as monoalkyl esters of long chain unsaturated fats began from normal oils and fats of plants and creatures, is a sort of option for non-renewable energy sources. Biodiesel has drawn in wide consideration on the planet because of its renewablity, biodegradablity, nontoxicity and harmless to the ecosystem benefits [16].

Biodiesel has drawn in wide consideration on the planet because of its renewablity, biodegradablity, non harmfulness and harmless to the ecosystem benefits. It is a significant new elective transportation fuel. It tends to be delivered from various feedstock containing unsaturated fats like creature fats, non eatable oils, and waste cooking oils and results of the refining vegetables oils and green growth. . [14]

Microalgae are considered as important bioresources because of their capability to collect protein sugars and other high-esteem metabolites inside the cell in view of their environmental specialty. Green growth can be considered as a feedstock for biofuels and other modern coproducts in light of taking off the energy cost, an unnatural weather change, and related decrease in normal sources. Microalgae-based item advancement is expensive because of energy cost and financial aspects related with the downstream cycles. Nonetheless, to support the monetary practicality of modern scale microalgal tasks, every one of the metabolites from biomass should be valorized in a biorefinery approach. High-esteem algal metabolite items for enterprises incorporate lipids, polyunsaturated unsaturated fats, shades, proteins, sugars, nutrients, and cell reinforcements and have possible applications in drugs, beauty care products, nutraceuticals, and biofuel businesses.[5] The cyanobacterial species in this study have poor literature reviews)about quality of oils and thus , this study aimd to focus on them for oil extraction and analysis of their fatty acidsto determin som important properties for algal biodiesel with made comparssion between them .

2.Methods of Experiment

2.1.Preparation of algal growth medium (Chu 10 medium) liquid

Chu 10 liquid medium modified for algal development was prepared with the proportions of the components shown below [17].

Material Weight (g/L)

Ca (NO3)2 0.4

K2HPO4 0.1

Na2CO3 0.2

MgSO4.7H2O 0.25

Na2SIO3 0.25

Ferric Ammonium Citrate 0.005

The components are dissolved in a liter of distilled water, then the pH of the medium is adjusted (7.6-7.8) using a pH-Meter and the pH value is equalized using dilute solutions of NaOH (0.1 N) and HCL (1 N). The prepared medium is distributed on the flasks with paper Aluminum is sterilized by an autoclave at a temperature of 121°C and a pressure of one atmosphere for 20 minutes [18].

2.2. Examination and development of the study sample

A laboratory sample of *Oscillatoria* algae (Scientific Education Research Unit for Pure Sciences/Algae Research) was used, where it was examined with a light microscope to confirm the thallus and the sample was photographed.

The algae were initially grown in glass flasks (the volume of 250 ml) containing a sterile medium (10 chu) and the modified liquid (5 ml / 100 ml of medium). Then, after a week of development, the inoculum was taken from it and transferred to simple photo reactors with a capacity of (1 liter). With the same percentage of the previous inoculation, the incubation was carried out at a temperature of 25±2°C and a lighting of 2500 lux, with air supplied with a filter (0.45 mm) and a blower gas, as shown in Figure (1).

Figure 1 :Making primary algae cultures and a simple photoreactor

2.3. Biomass estimation

A sample of the algae was taken after centrifugation (3000 rpm for 20 minutes) for the liquid medium cultured with algae. The fresh weight was measured using an empty Petri dish and with a sensitive balance. The sample was transferred to an oven at a temperature (30-40°C) for two days and then recorded. The dry weight of the sample and the dry mass of the moss was collected in a sterile airtight container until extraction [19].

2.4. Extracting lipids from algae

Dry moss mass was used, after crushing with a small ceramic mortar, the dried moss powder was placed in a Whatman 1 filter paper and formatted in such a way as to form a cellulose thimble (Soxhelet) [20].

2.5. Estimation of oil yield

This is done by calculating the weight of the algae powder and calculating the weight of the oil produced from algae after oil extracting from algae by soxhlet [21].

2.6. Separation of fatty acids

2.6.1. Soaping process:

Use a KOH solution (7.5 M) with the fixed oil extracted from the algae by adding it in a ratio of (10:1 v/v) and heat escalation for 90 minutes at a temperature of 100 ° C, then the solution is cooled to the laboratory temperature and the same volume of the added KOH solution is added to it after Cooled down to form an emulsion, then transferred to a separation funnel and 15% (twice) air was added to remove the unsolved fat. H_2SO_4 (sulfuric) acid is added at a concentration of 20% until we get a pH = 2, then the solution is returned to the separating funnel to extract the fatty acids from it by adding ether at 25% (twice). Filtering and using a rotary evaporator, it is concentrated and kept in the refrigerator [22].

2.6.2. Esterfication of fatty acids

In this process, the methyl group of the fatty acid is added after the soaping process to convert it to a less polar state and increase its volatility when using GC technology.

0.5 ml of separated fatty acid was taken, 0.1 ml of acetyl chloride and 25 ml of methanol were added to it. The solution was transferred to a water bath at a temperature of (100 °C) for 20 minutes, then the solution was left to cool at room temperature and kept in the refrigerator until using the GC device to diagnose the type of infection. fatty acids in it.

Diagnosis of fatty acids using Gas Chromatography GC

The GC technique was used to diagnose the separated fatty acids from the Oscillatoria oil sample after soaping and esterification, and standard fatty acids were used in order to depend on the Retention Time (Rt) (min) to diagnose the fatty acids under study.

Standard fatty acids were prepared by dissolving 0.1 g in 10 ml of ether and these are: Palmitic acid, Linolelaidic , Stearic, Oleic , Linoleic , butyic , myristic , etc (Table 2).

The examination was carried out in the laboratories of the Department of Environment and Water / Ministry of Science and Technology using the GC device shown in (Figure. 2) with the specifications:

- Capillary column type (SE-30) with lengths (30mm*0.25mm*0.25mm).

-FID ionized flame detector.

- SHEMDZU, 2010.

- Japan.

- The temperature of the injection area is 330 °C and the temperature of the detector area is 280 °C. The temperature of the lobe column is gradual, starting from 120-280 °C, with a rise rate of 8 °C/min.

- Carrier gas is inert nitrogen gas with a rate of 100 KPa.

The percentage of each fatty acid was recorded according to the measurements of the device data, which are shown later in the results.

Determination for some biodiesel features

There are many features of biodiesel and the importance between them;

DU-Degree of Unsaturation

DU = MUFA + (2 X PUFA)------[23]

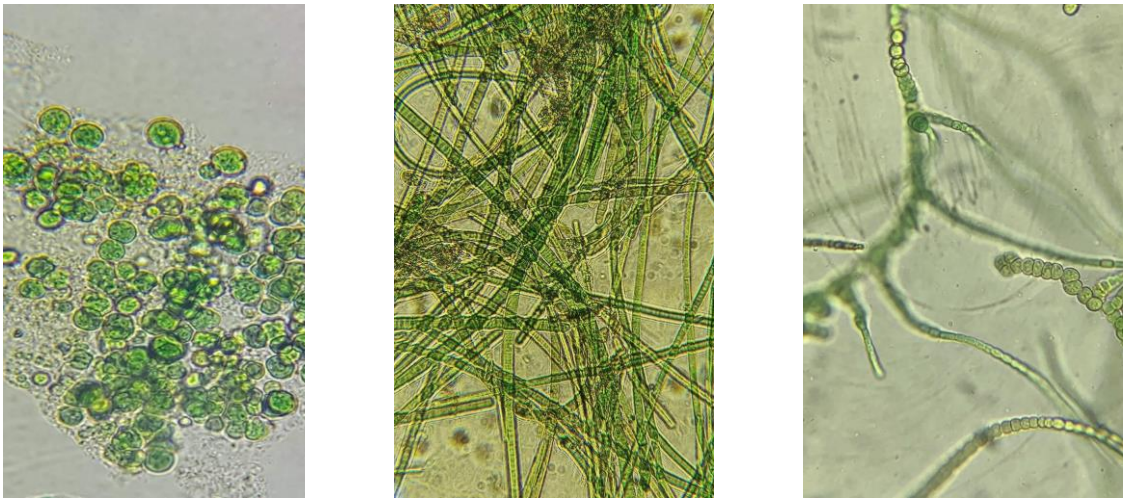
CN- Cetane Number:

CN= 62.32 – 6.13 . DU ------ [24]

Figure 2: GC Chromatography device (Shimadzu 2010)

3.Results

In this study we used different types of algal thallus, *Gloeocapsa*- unicellular cyanobacteria , *Oscillatoria*- simple unbranched filaments while *Westiellopsis* is branched filamentous thallus (Figure 3).



Gloeocapsa *Oscillatoria* *Westiellopsis*

Figure 3: Microscopic view for Cyanobacterial Isolates

Dry biomass collection

The dry algae biomass was collected after drying the soft biomass in the Oven at 40°C for 24 hours and kept in sterile airtight containers until use(24).

Algae oil extraction

Use each time to extract with a Soxhlet continuous extraction device, a dry weight of 1-5 gm of dried algae was used upon alga species. After completing the extraction process (about 10 hours), the algae oil was obtained into the crude, from which the solvent (hexane) was evaporated. Later, a rotary evaporator was used to obtain the final oil sample. Which will be diagnosed for its content of fatty acids later. Keeping the oil in tightly closed glass bottles (Figure 4). The percentage of oil yield is estimating according to the following equation (%Oil yield = weight of Oil extract / weight of Dried algae biomass taken × 100) [21].(Table 1)

Figure 4: Algal Oil

Table 1: Yield of algal oils

Sample No.	Alga Species	Dry Weight of alga	% Lipid Yield
1	*Gloeocapsa*	**3**	**18.5**
2	*Oscillatoria*	**5**	**6.1**
3	*Westiellopsis*	**1**	**20**

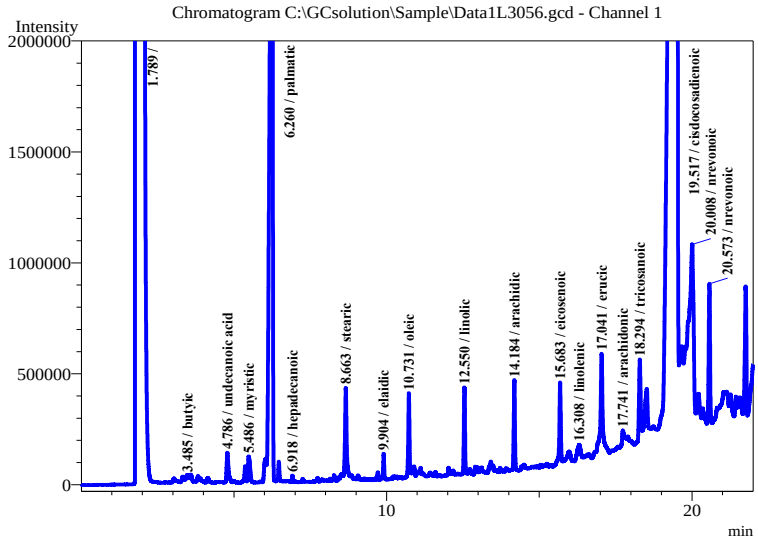
The results in table 1 showed the % of oil extracted from algal species , the algae *Westiellopsis and Gloeocapsa* have almost similar values 20 and 18.5 % respectively ,while the alga *Oscillatoria* % oil is 6.1% .

Determination of fatty acid composition by GC

The obtained data after detection of algal oils fatty acids by GC device ,that compared with standard fatty acids retention time as in Figure 5 , refers to quantity and quality of each algal oil (Figure 6 A,B,C) and this data arranged with groups and information for each fatty acid type (Table 2) .

Sample Information

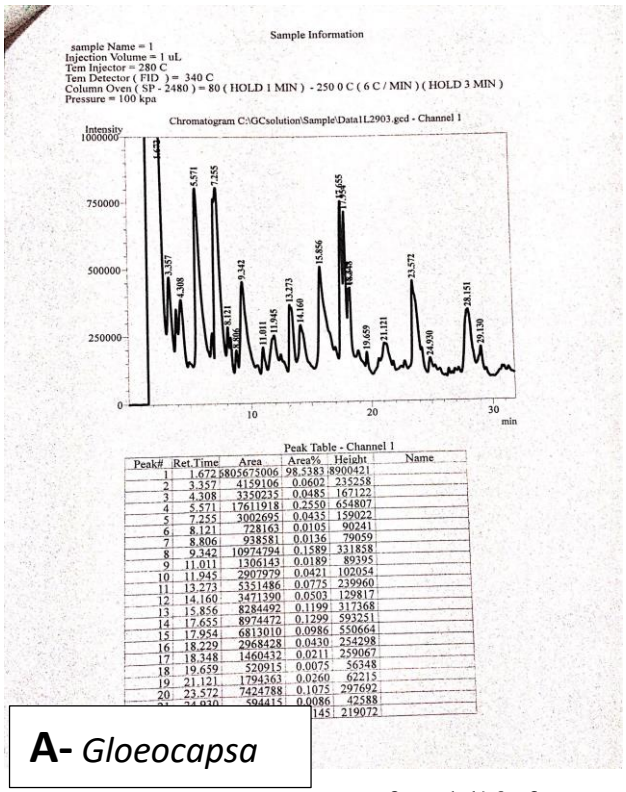
Sample Name = FAME 0.5 %
Injection Volume = 1 uL
Tem Injector = 280 C
Tem Detector (FID) = 340 C
Column Oven (ZB-5) = 120 C (HOLD 2 MIN) - 300 C (10 C / MIN) (HOLD 2 MIN)
pressure= 100kpa



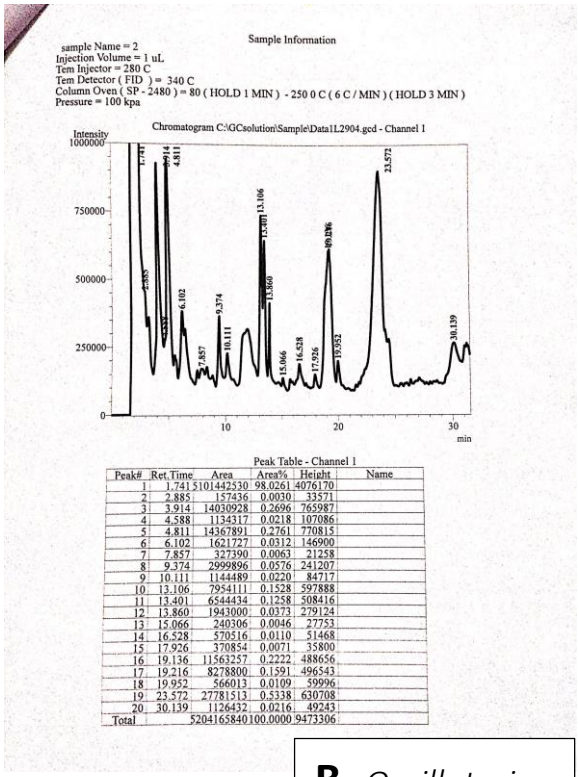
Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Name
1	1.789	3757736973	96.5758	479923849	
2	3.485	78248	0.0020	17354	butyic
3	4.786	446524	0.0115	123856	undecanoic acid
4	5.486	498134	0.0128	111467	myristic
5	6.260	24476180	0.6291	4119974	palmatic
6	6.918	53932	0.0014	27438	hepadecanoic
7	8.663	1139321	0.0293	366464	stearic
8	9.904	283710	0.0073	113578	elaidic
9	10.731	1006479	0.0259	366429	oleic
10	12.550	841200	0.0216	351995	linolic
11	14.184	1099883	0.0283	404266	arachidic
12	15.683	1081835	0.0278	355923	eicosenoic
13	16.308	469989	0.0121	64080	linolenic
14	17.041	1863019	0.0479	425211	erucic
15	17.741	341981	0.0088	61968	arachidonic
16	18.294	1261425	0.0324	346168	tricosanoic
17	19.517	91285767	2.3461	7436458	cisdocosadienoic
18	20.008	5090487	0.1308	630853	nrevonoic
19	20.573	1915554	0.0492	625830	nrevonoic
Total		3890970641	100.0000	495873161	

Figure 5: GC Data for standard fatty acids

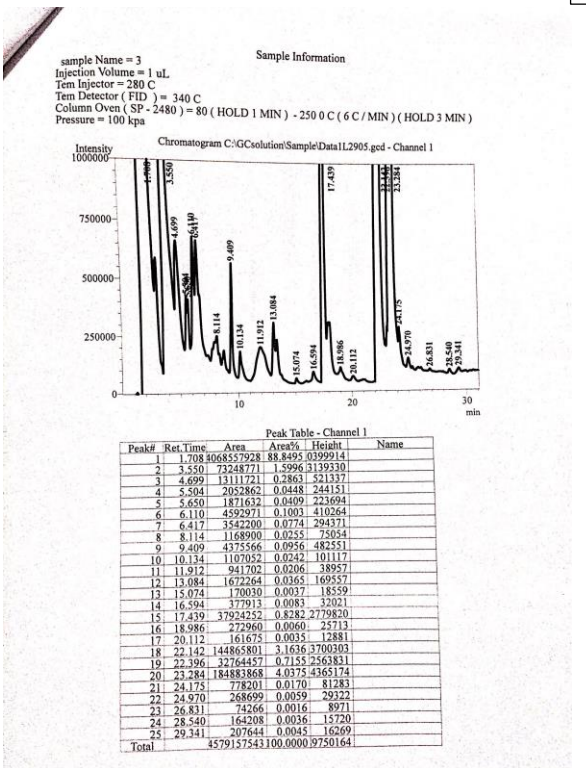


A- *Gloeocapsa*



B- *Oscillatoria*

Scanned with CamScanner



C- *Westiellopsis*

Figure 6: GC Data for algal oils fatty acids

Table 2: Algal oils fatty acids components

No.	Fatty acid	Formula	*Gloeocapsa*	*Oscillatoria*	*Westiellopsis*
1	Butyric acid	C 4:0	0.060	0.270	1.600
2	Undecanoic acid	C 11:0	0.049	0.276	0.286
3	Myristic	C 14:0	0.255	ND	0.045
4	Palmitic	C 16:0	ND	0.031	0.100
5	Heptadecanoic	C 17:0	0.044	ND	0.077
6	Stearic	C 18:0	0.014	0.006	0.026
7	Elaidic	C 18:1	0.159	0.058	0.096
8	Oleic	C 18:1	0.019	0.022	0.024
9	Linolic	C 18:2	0.042	0.153	0.021
10	Arachidic	C 20:0	0.050	0.037	ND
11	Ecosenoic	C 20:1	0.120	0.005	0.004
12	Linolenic	C 18:3	ND	0.011	0.008
13	Erucic	C 22:1	0.130	ND	ND
14	Arachidonnic	C 20:4	0.099	0.007	0.828
15	Tricosanoic	C 23:0	0.043	ND	0.006
16	Cisdocosadienoic	C23:2	0.008	0.159	ND
17	Nervonic	C 24:1	0.026	0.011	0.004
18	Nervonic	C24:1	ND	ND	ND
∑1	Total (C4-C24) S.+Unsa. F. A.		1.118	1.046	3.141
∑2	Saturate F. A.	SAFA/C.:00	0.515	0.62	2.14
∑3	Unsaturate F. A.	USFA/C.:1-4	0.603	0.426	0.985
∑4	Monounsaturate F. A.	MUFA/C.:1	0.454	0.096	0.128
∑5	Polyunsaturate F. A.	PUFA/C.:2-4	0.149	0.330	0.857
∑6	Non Identified F.A.		0.342	0.940	8.014
∑7	Sat.and Monounsa.F.A.	SAFA+MUFA	0.969	0.716	2.268
∑8	Total F. A.		1.460	1.986	11.139

The values in table 2 and Figure 7,8 shows the fatty acids composition of the oils extracts from the species of algae .The predominant FA which interest for biodiesel quality were Palmitic acid C16:00 , Oleic acid C18:1, Linolic acid C18:2 and Linolenic C18:3.

Gloeocapsa and *Oscillatoria* shows equilibrium SAFA value (0.515% and 0.62%) respectively , while *Westiellopsis* became superior on them (2.14%).As well as, the values of USFA , but there are clear different in % MUFA and PUFA . *Gloeocapsa* have the best MUFA %(0.454) which with SAFA make (0.969% of favorable FA for best biodiesel quality, whereas *Westiellopsis* have increased of it (2.268%) but with high non identified FA and high PUFA which is result in non desired quality for biodiesel.

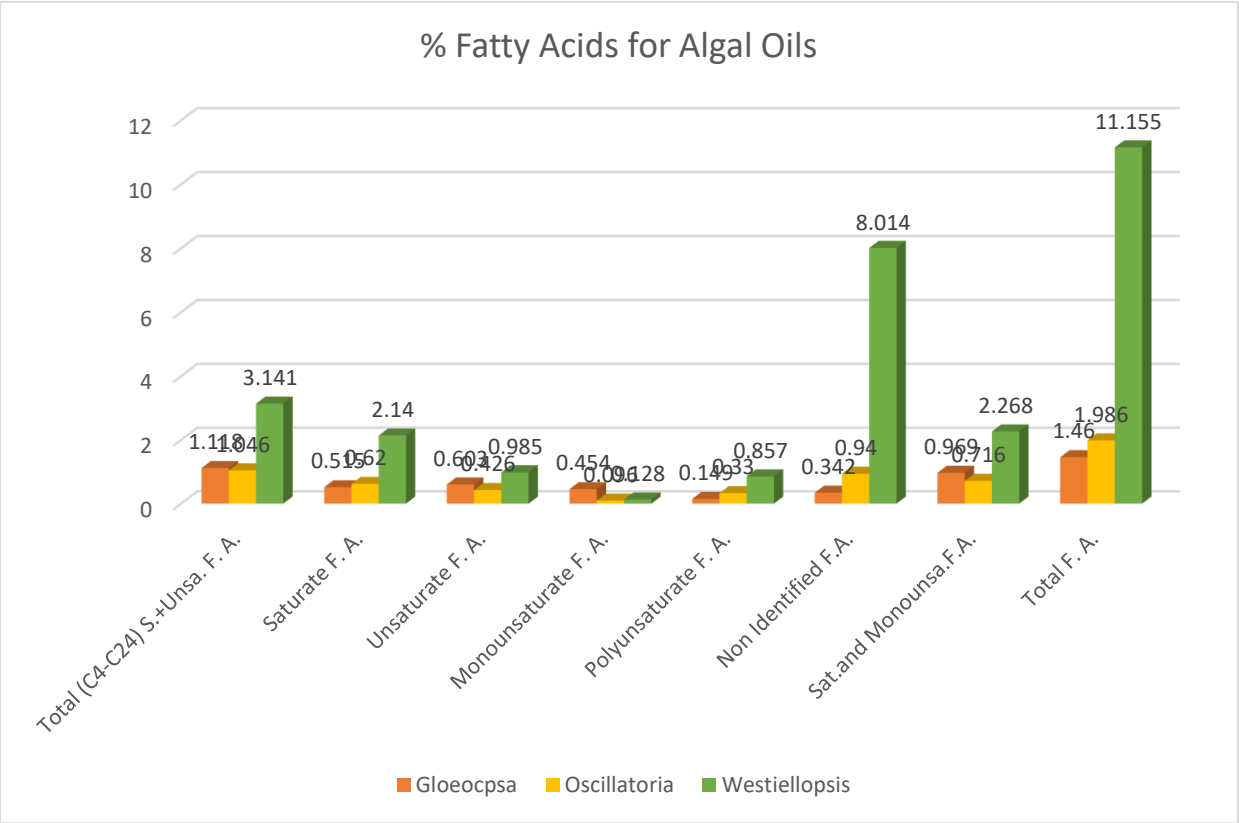


Figure 7: Algal FA components from GC data

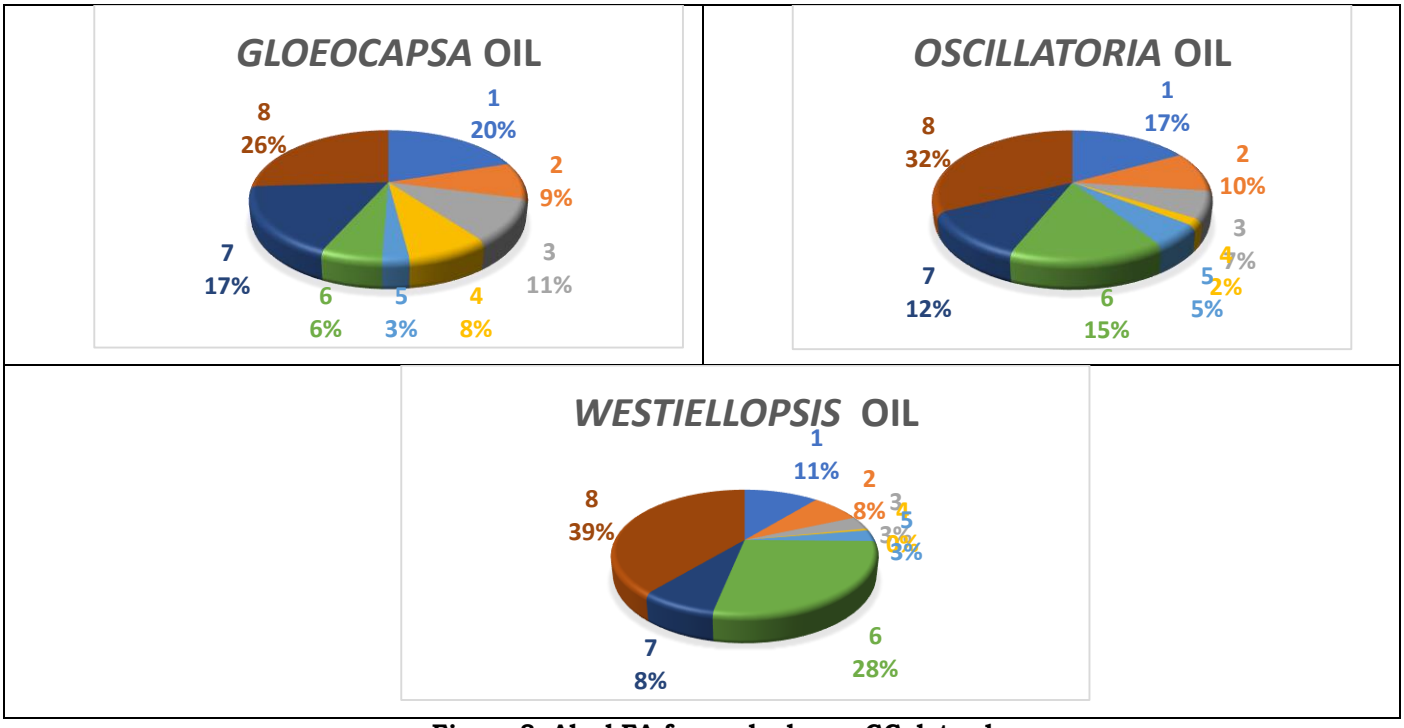


Figure 8: Algal FA for each alga as GC data shows

Determination of some quality features of biodiesel

Biodiesel quality affected strongly with quality and quantity of oil FA, thus from GC data for FA detection there is benefit in choosing best oil quality for adopting it as a source of biodiesel. Some of these features (Table 3) are documented from GC data as % for FA and other calculated from equilibriums as later mention to it in Materials and Methods

Table 3: some quality features of algal biodiesel

NO.	Algal name	USFA/SAFA (%)	O/L(%)	DU	CN
1	*Gloeocapsa*	1.171	0.452	0.752	57.77
2	*Oscillatoria*	0.690	0.144	0.756	57.69
3	*Westiellopsis*	0.460	0.875	1.842	51.03

O/L=Oleic %/Linoleic %

DU= Degree of Unsaturation

CN= Cetane Number

Data in table 3 refers to highest USFA in *Westiellopsis* that results in highest DU with corresponding to lowest % USFA/SAFA as well as lowest CN, while the equilibrium of DU for *Gloeocapsa* and *Oscillatoria* showed owning an equilibrium CN too.

4. Discussion

The quality and quantity of fatty acids in algal lipids and any other oil represent an important point that effect on the produced biodiesel from these oils. Thus, in this work the start point to select algal species for high biodiesel production is detecting oils fatty acids.

Usually , saturated fatty esters have high cetane number and superior oxidative stability,whereas unsaturated , PUFA esters have improved low temperature combustion properties.The value obtained for the concentrations of SAFA, MUFA and PUFA for microalgae *Chlorella vulgaris* and others showed comparison in FA component [24].One of important feature of biodiesel CN , standards for CN indices for ASTM D6751 requires a minimum CN of 47, while in the European standard EN14214 is 51. The values obtained for different microalgae species range from 52.2 to 56.7 and are in accordance with all standards reported[25].In this study CN as showed in table 3 ,it ranged in values of literatures above mention to it.

The MUFA and PUFA productivity. Is higher ,resulting in higher quality lipids for biodiesel production and the PUFAs existing in lipid may decrease the biodiesel stability. Higher salinity is reason for PUFAs decreasing [26]. Fatty acids like C16;0, C18;0, C18;1 and C18;2 are primarily responsible for the quality of biodiesel made from FAME. The ideal form of lipid for the synthesis of biodiesel is one with a greater percentage of C16-18 since it produces a higher cetane number and more heat of combustion [27].

Contrary to macroalgae, which often create sugars and other carbs instead of lipids, microalgae can produce large amounts of lipids, which are the building blocks of the manufacture of biodiesel [28]. On the other hand, [29] makes the argument that there may be chances for algae biodiesel production to be more economically feasible when it is integrated with other processes, such as wastewater treatment, although this does not guarantee large-scale production.

Microalgae and algae produce biodiesel mainly composed of PUFA . The unsaturation level affects the oxidative stability , ignition quality and flew properties .

Many of the algal profiles contain substantial amounts of highly unsaturated species including FAs with 3-6 double bonds [30] .

5.Conclusion

Algae is very important as an exporter of biomass and it will be the most suitable source for different types of fuel, in addition to that its development takes place during the purification of the environment and the recycling of primary elements to convert them into basic compounds for the production of types of renewable energy from a renewable and sustainable source. Algae Growth, including microalgae cyanobacteria, during their growth, produced materials and energy and reduced pollution in the environment. Environmentally friendly fuels were obtained from them, in addition to other compounds and enormous uses in various fields that serve humanity and all organisms. It is actually as the developed countries of the world call it green gold.

Algae utilize CO_2 for their growth and can minimize some sort of pollution level and result in carbon credit for a country .In general algae can be grown away from farmlands and forests and their yields of oil are orders of magnitude higher than those from traditional oilseeds. In my country and most areas algae are growing in many water bodies as well as terrestrial humid places but it suffering from neglect and waste . Algae from that perspective are very promising resource for the production of biodiesel . Cyanobacterial production could become economically feasible in the future when biotechnical, Environmental and economic hurdles will be surmounted . The properties of algal oil vary depending on the type of algae, and excellent ratios of saturated and monounsaturated fatty acids appeared, which are good donors for the good properties of the biodiesel derived from them, As well as the presence of different degrees of polyunsaturated fatty acids, and thus it is very useful to diagnose the quality and quantity of fatty acids of algae oil before adopting it as a source of biodiesel.

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